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Reactive Site Binding

FIG. 1A

Chemical reaction to be catalyzed

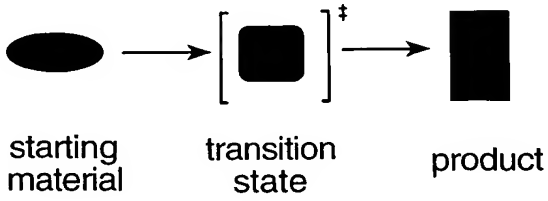


FIG. 1B

PROfusion™ affinity binding to transition state analog

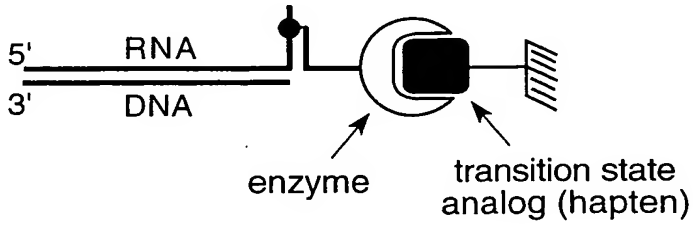
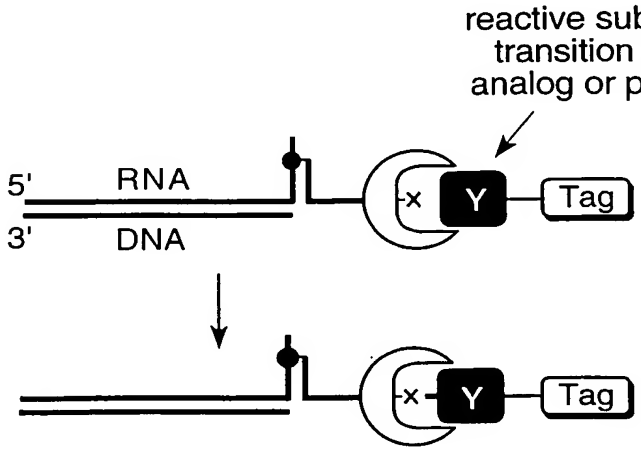


FIG. 1C

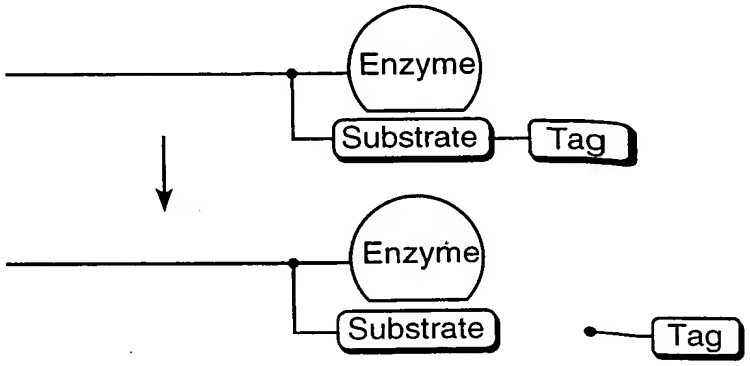
Covalent binding to reactive substrate, transition state analog, or product



Enzyme-Substrate Chimeras

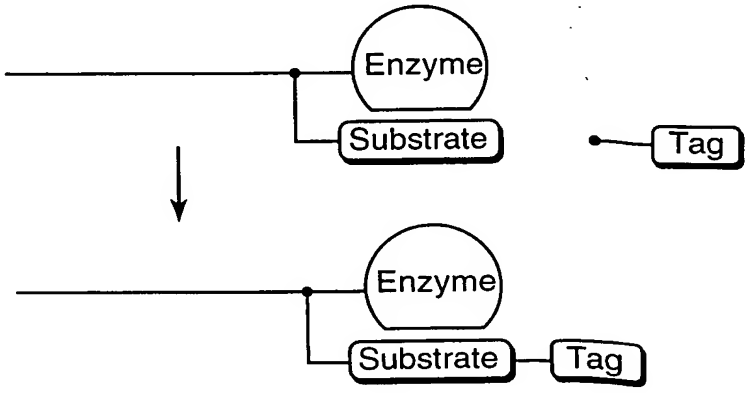
FIG. 2

1. Mono-molecular reaction



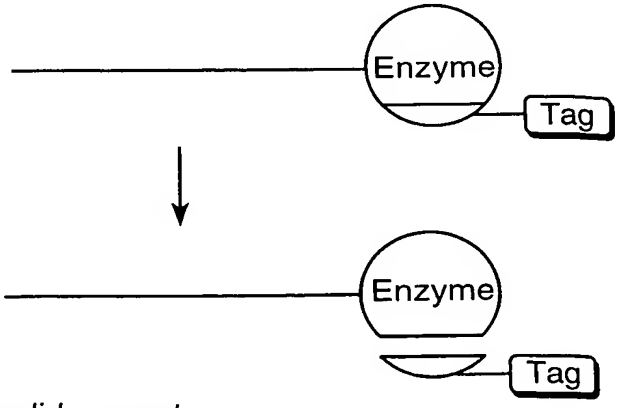
Cleavage from tag or solid support

2. Bi-molecular reaction



Attachment to solid phase, reaction with biotinylated substrate followed by capture on streptavidin resin, product immunoprecipitation with suitable antibody or gel-electrophoretic separation of modified and unmodified fusion (or cDNA portion)

3. Autoproteolytic

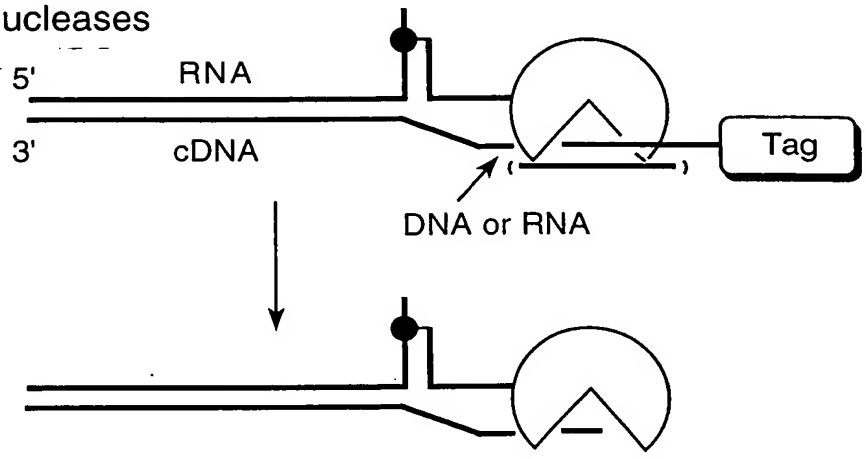


Release from tag or solid support

Nucleases

FIG. 3

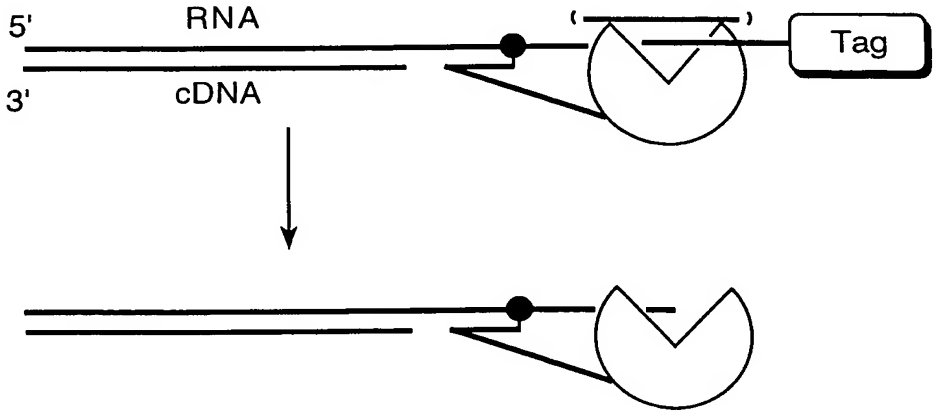
- Desoxyribonuclease
- Ribonuclease
- Restriction endonucleases



RNA-protein fusion

~~PROfusion~~™ DNases or endonucleases promote their self-cleavage from a tag or solid support. The use of the second strand is optional. Sequence-specific cleavage can be achieved through the choice of the target sequence. Similarily, this method can be used to alter the restriction site specificity of restriction enzymes after mutagenesis.

- Ribonuclease



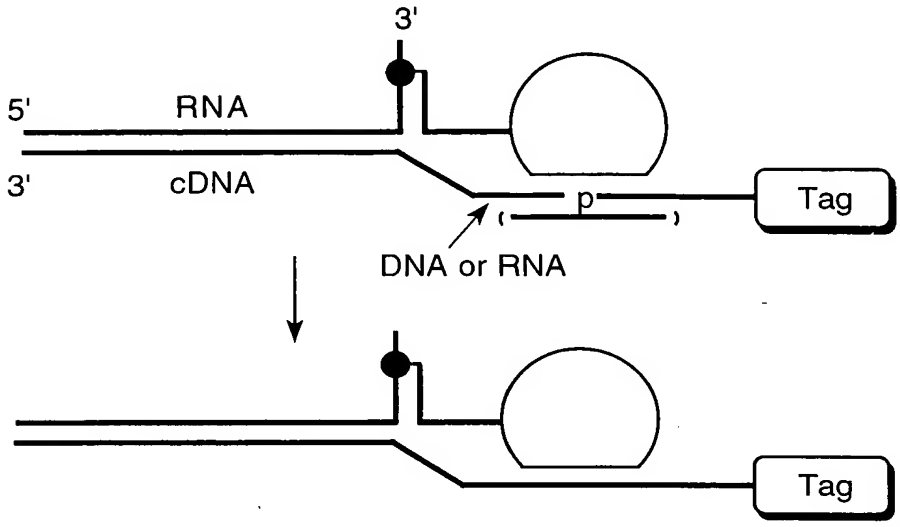
RNA-protein fusion

~~PROfusion~~™ DNases promote their self-cleavage from a tag or solid support. The use of the second strand is optional. Sequence-specific cleavage can be achieved through the choice of the target sequence.

Ligases

FIG. 4

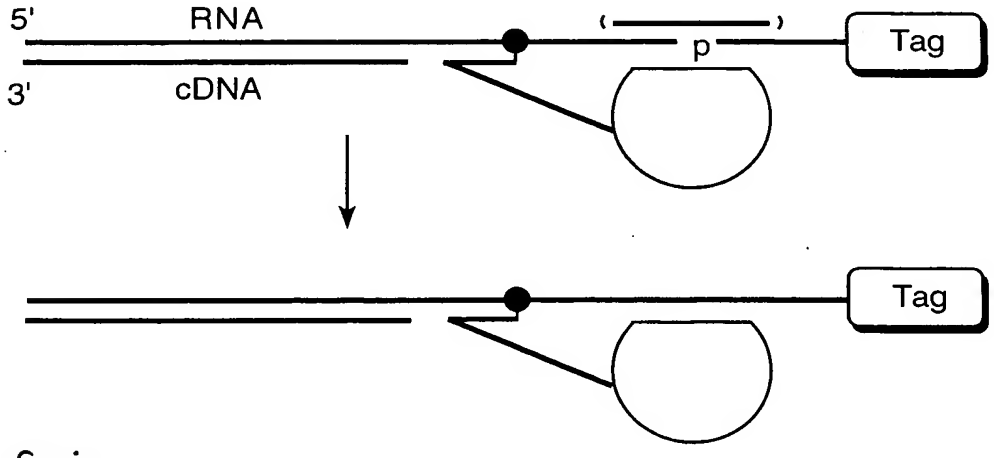
- DNA ligase
- RNA ligase



RNA-protein fusion

~~PROfusion~~™ DNases or RNA ligases catalyze their attachment to a tag or solid support. The use of the second strand is optional. Sequence-specific cleavage can be achieved through the choice of the target sequence. The second substrate is either directly attached to the solid phase, or e.g. biotinylated to allow capture with immobilized streptavidin. Alternatively, the size-difference between precursor and product may be used for electrophoretical separation.

- RNA ligase



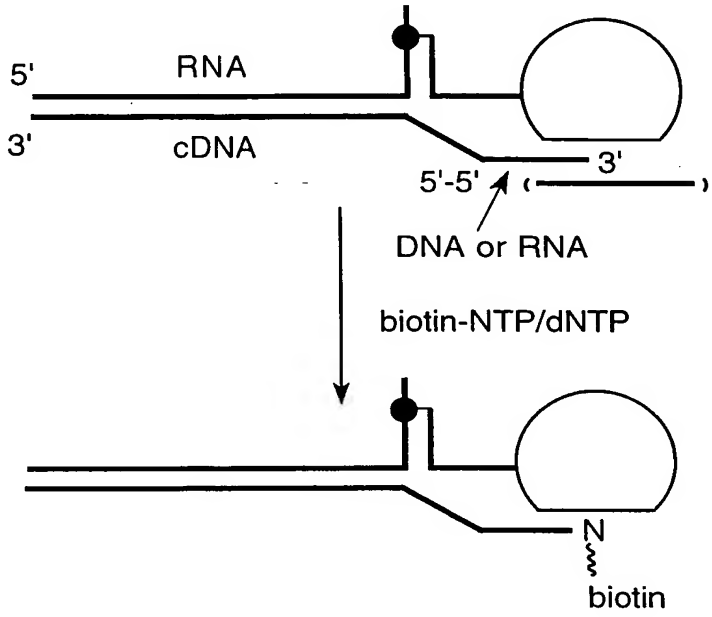
RNA-protein fusion

~~PROfusion~~™ RNA ligases catalyze their attachment to a tag. Similar considerations as for DNA ligases apply.

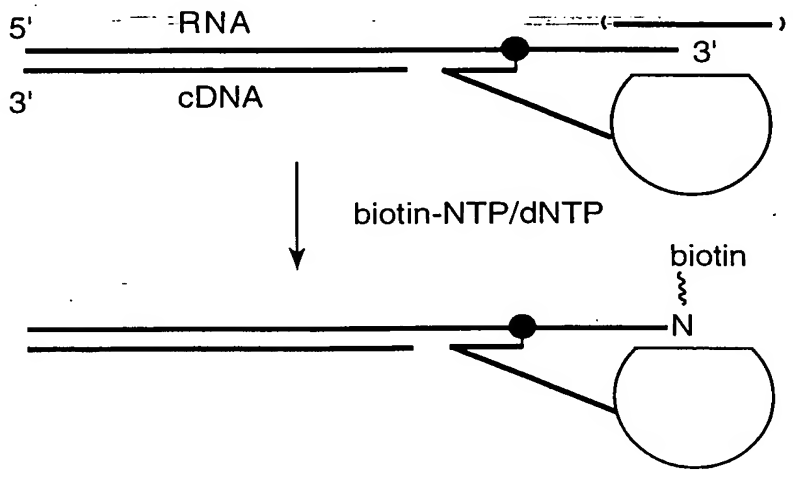
Polymerases and Terminal Transferases

FIG. 5

- Terminal transferase
- DNA polymerase
- RNA polymerase
- Reverse transcriptase



- Terminal transferase
- RNA polymerase
- Reverse transcriptase



RNA-protein fusion

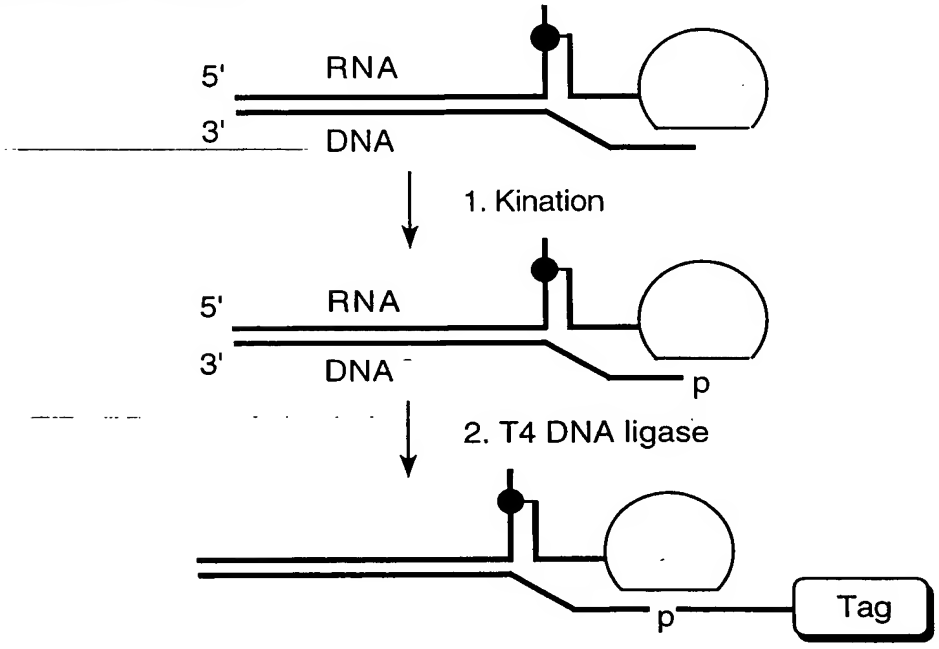
~~PROfusion~~™ capture through attachment of biotinylated nucleotide triphosphates. For the selection of polymerase enzymes a second strand must be used. Following reaction, the modified ~~PROfusion~~™ can be captured with streptavidin resins.

RNA-protein fusion

Kinases and tRNA Synthetases

FIG. 6

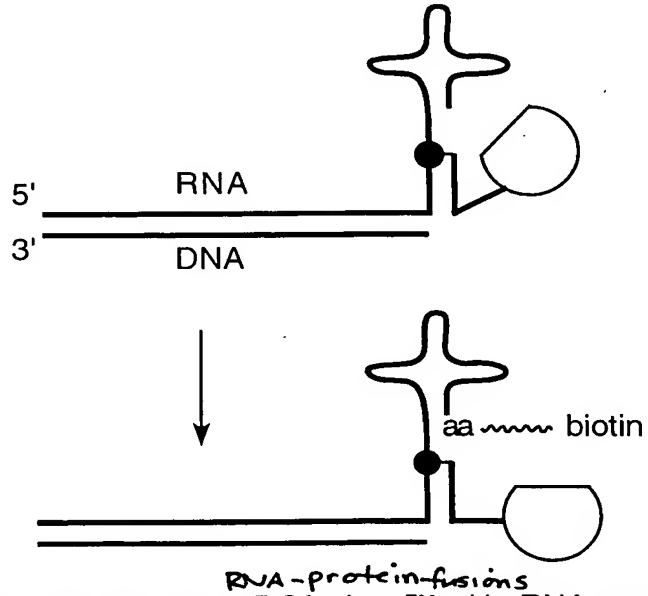
- Polynucleotide Kinase



RNA-protein fusions

After phoshorylation, the kinase ~~PROfusions™~~ become substrates for ligation to allow the physical separation from the unmodified precursor.

- tRNA synthetase



RNA-protein fusions

Attachment of biotinylated amino acids through ~~PROfusions™~~ with tRNA synthase activity. Successfully modified molecules may be captured on streptavidin supports. Note that the tRNA domain may also be attached to the cDNA portion.

Substrate Attachment

FIG. 7A

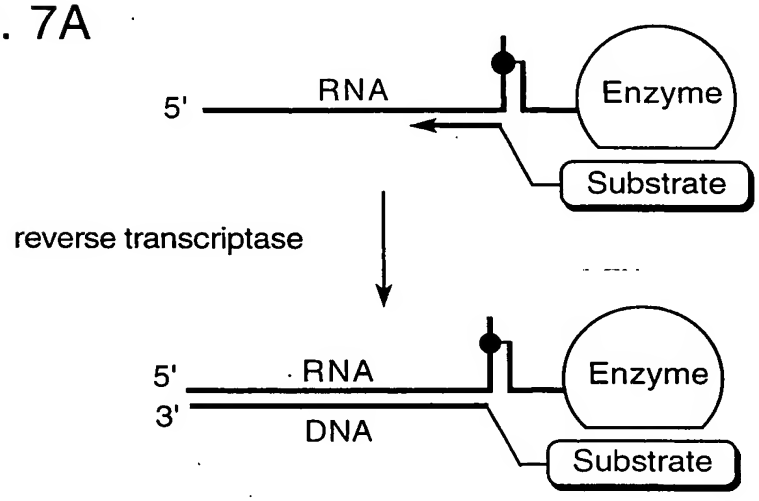


FIG. 7B

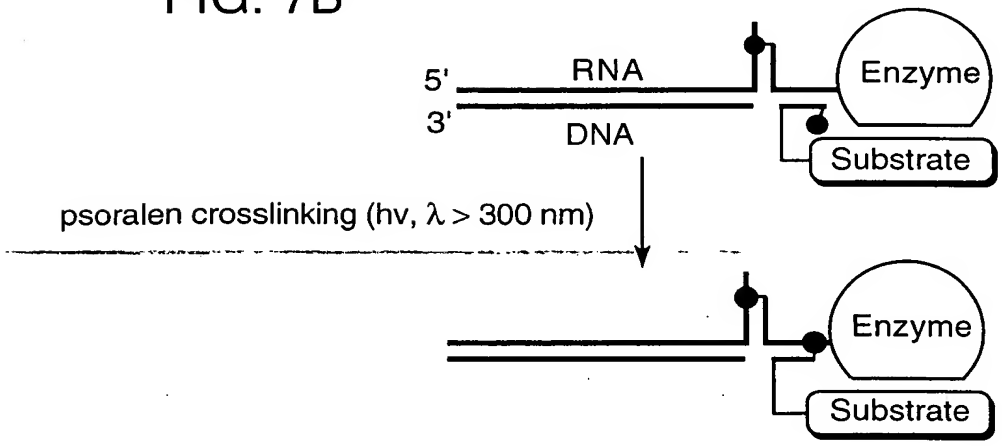


FIG. 7C

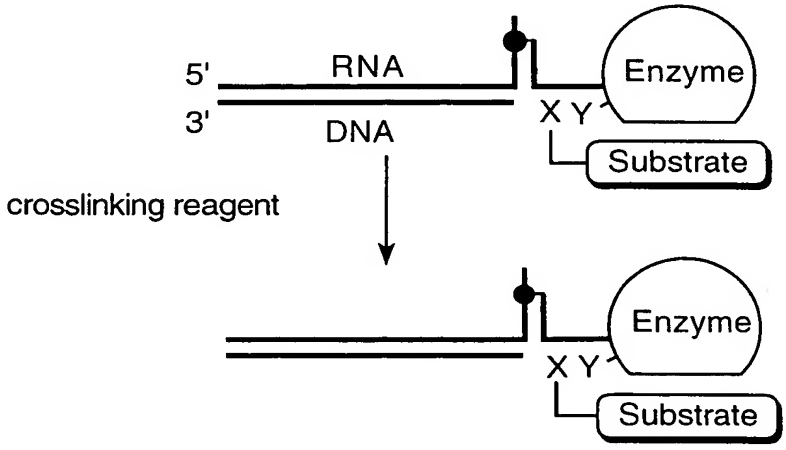
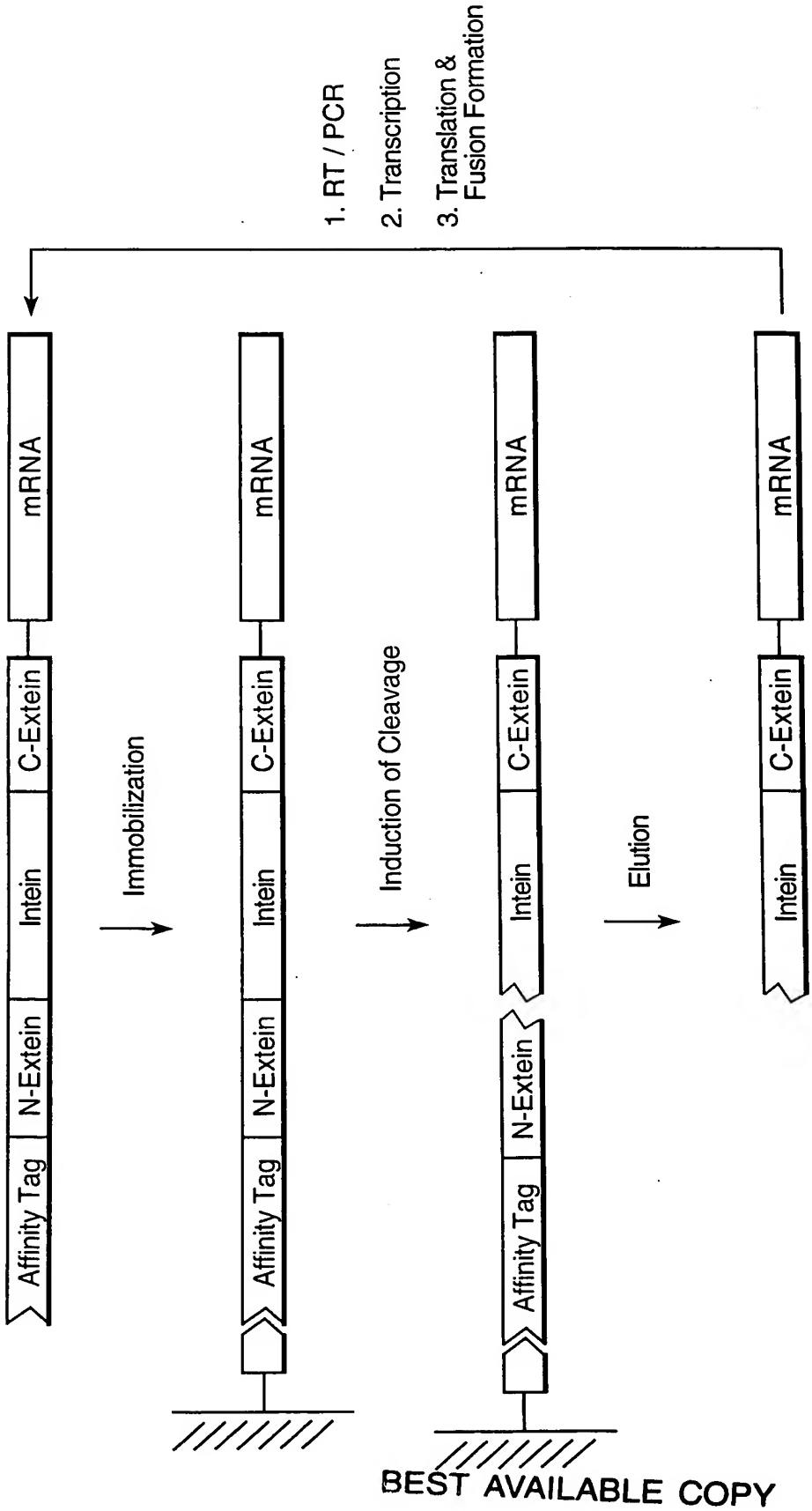


FIG. 8



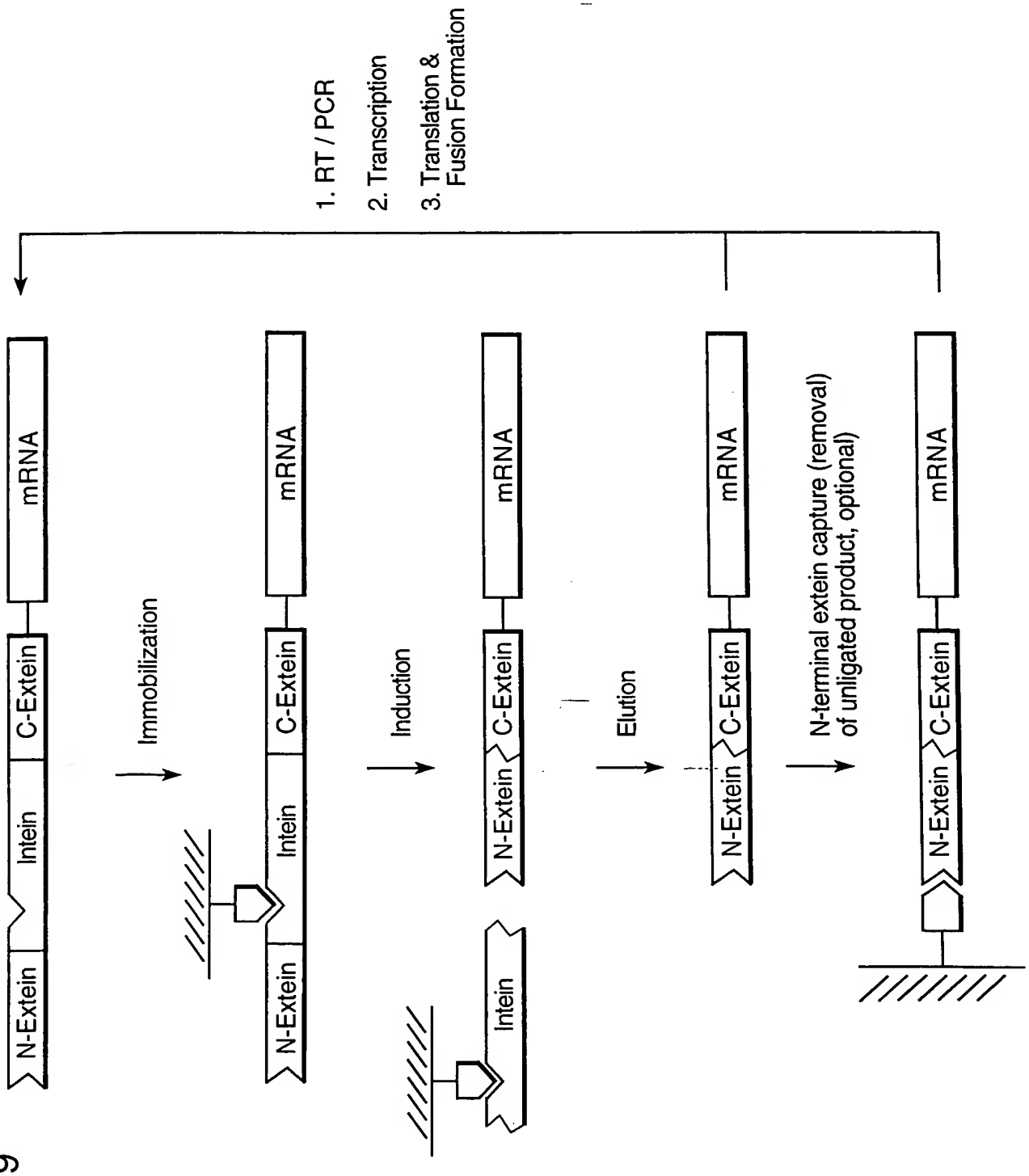


FIG. 9